Flavor Studies on Beef and Pork

The odor responses, and the chemical compounds isolated, from the volatile pyrolysis products of lyophilized cold water extracts of lean beef and lean pork were found to be similar. The flavor precursors in lean meat are low molecular weight compounds present in the dialyzable portion of the cold water extracts of the raw lean meats. Beef and pork fat when heated produced dissimilar aromas. Free fatty acids and carbonyls were determined in these fats before and after heating. The results suggest that, on heating, the lean portions of pork and beef contribute an identical meaty flavor to these meats, while the characteristic flavor differences in pork and beef reside in the fat.

PRIMARY EMPHASIS has been placed on the odor constituents of meats. The authors previously studied lean beef and found the flavor precursors present in the raw meat to be cold water–extractable. This extract was lyophilized, and the resulting powder heated under vacuum and fractionated into two major portions. The more volatile fraction has been studied (*7*). Investigation of the less volatile fraction (fraction I) is reported in this paper. Lean pork has also been subjected to the techniques previously applied to lean beef and its volatile constituents have been examined. In addition, beef and pork fat have been analyzed for free fatty acids and monocarbonyl compounds, and possible flavor precursor systems have been studied.

Experimental

Lean Beef, Fraction I. The powder obtained by the lyophilization of a cold water extract of raw, lean beef contains flavor precursors of cooked beef. The total volatiles produced by pyrolysis of this powder at 100° C. are trapped at liquid nitrogen temperatures and fractionated at room temperature under vacuum into two major fractions. The less volatile of these two fractions, fraction I, is a viscous residue of meaty aroma (*7*).

INITIAL OBSERVATIONS. Approximately 100 mg. of fraction I were obtained for every 30-gram batch of dried powder pyrolyzed. The pH of a water solution of 10 mg. of I per ml. varied from 3.5 to 4.0. The infrared spectrum of a film of I on rock salt plates was obtained on a Perkin-Elmer Model 137 Infracord spectrophotometer. Major peaks were recorded at 3.15, 5.81, 6.32, 7.12, 8.9, 9.6, and 11.7 microns (Figure 1). The ultraviolet spectrum of a water solution of 2 mg. of I per ml. was obtained on a Beckman DU spectrophotometer; a maximum was observed at 290 to 295 mμ. Elemental analysis of I was: C, 34.21%; H, 7.69%; N, 5.46%; S and P, absent; and O (by difference), 52.64%. The neutral equivalent of I was 216.

PAPER CHROMATOGRAPHY OF I. Fraction I was best separated on paper by developing the chromatogram with butyl alcohol saturated with water. The ascending chromatographic technique and apparatus described by Mitchell (*12*) were used to separate I on a milligram scale. Two Whatman No. 1 sheets, 8 × 8 inches, were streaked across the paper 1 inch from the bottom with 2 ml. of methanol containing 37.5 mg. of I, and the chromatogram was developed until the solvent front was 1 inch from the top. The papers were hung in a well ventilated hood to dry, and then a 0.5-inch strip was cut from the edge of the sheet and sprayed with 1% permanganate. Three bands appeared at R_f 0.82, 0.50, and 0.25. The remainder of each of these fractions was eluted with methanol, concentrated on a rotary evaporator, rechromatographed, and again eluted with methanol. An appropriate amount of each solution was placed on a rock salt plate and the solvent volatilized by heat. The infrared spectra for the fractions at R_f 0.82 and 0.25 were obtained. An insufficient amount of material of the components at R_f 0.50 was recovered to obtain an infrared curve. Ultraviolet spectra of water solutions containing 2 mg. per ml. of the fractions recovered at R_f 0.82 and 0.25, respectively, gave no characteristic peaks; when 3 ml. of water were added to the residue from the fraction at R_f 0.50, a slight increase in absorption at 290 to 295 mμ was observed.

TITRATION CURVES. Seven milliliters of the solution to be titrated, containing 77.0 mg. of fraction I, were placed in a small constant temperature cell equipped with electrodes attached to a line-operated pH meter. The solution was stirred magnetically and blanketed by nitrogen. A calibrated micropipet, made from a glass capillary and a vernier micrometer, was used to add small increments of alkali or acid. After each addition, the pH of the solution was recorded.

LABILE NITROGEN. Labile nitrogen in I was determined by comparing Kjeldahl nitrogen results with those obtained by the following modified Kjeldahl procedure. Twenty-five milligrams of I in 3 ml. of water were placed in a test tube ending in a standard-taper 24/40 joint connected to an adapter, through which 5 ml. of 30% sodium hydroxide were added and through which nitrogen gas was admitted. The nitrogen passed slowly over the magnet-

ically stirred solution and into the trapping solution (*11*). The ammonia collected after 24 hours was determined by titration with 0.01*N* hydrochloric acid.

Optical Activity. The optical rotation of I was measured by means of a Keston spectropolarimeter attachment (Keston polarimeter Model D, Standard Polarimeter Co., New York, N. Y.) used with a Beckman Model DU spectrophotometer.

Determination of Lactic Acid. Lactic acid in I was determined by the method of Hullin and Noble (*8*).

Lean Pork. The techniques developed for lean beef (*7*) were applied to lean pork. Fat was removed as completely as possible from a cut obtained from a carcass kept at 0° to 4° C. for one week. The lean pork was ground, extracted with water, centrifuged, and filtered, and the water extract lyophilized to yield a powder containing 3% by weight of the starting material. Thirty grams of the freeze-dried pork extract were carried through the vacuum pyrolysis procedure devised for beef (*7*). The total volatiles trapped at −195° C. were fractionated under vacuum at room temperature to yield, as in the case of beef, a volatile fraction of an ammoniacal sulfurous odor and a nonvolatile fraction, pork I, of a fruity odor that turned to a meaty odor on exposure to air. The infrared spectrum of a film of pork I is shown in Figure 1. The ultraviolet spectrum of a water solution containing 2 mg. of pork I per ml. had an absorbance peak at 290 to 295 mμ. Pork I was chromatographed using techniques applied to I obtained from beef. Labile nitrogen and lactic acid were also determined.

The most volatile fraction obtained from pork was analyzed for carbonyls and acidic and basic components, using the procedures described in (*7*).

Beef and Pork Fat. Determination of Free Fatty Acids in Beef and Pork Fat. Depot fat was stored in a deep-freeze at −40° C. and samples were taken as needed. The free fatty acids and their concentration in the fat were initially determined. Twenty grams of fat were heated on a steam bath under nitrogen until molten, then filtered through several folds of cheesecloth. Ten ±0.2 grams of this filtrate were weighed into a 250-ml. Erlenmeyer flask and 30 ml. of petroleum ether added. The free fatty acids were separated from the fat by adsorption on an anion exchange resin, converted to their methyl esters, and identified and determined by gas chromatography, using the procedure and operating parameters described by Hornstein *et. al.* (*5*). The results are given in Table I.

The change, in kind and amount of free fatty acids present in fat, induced by heating under vacuum at 100° C., was studied. Twenty grams of fat were carried through the vacuum pyrolysis procedure (*7*). The total volatiles, however, were not further fractionated; instead, 10 ml. of water were added and the mixture was titrated directly with 0.01*N* sodium hydroxide. No titratable acidity was found. The residue in the pyrolysis chamber was analyzed for free fatty acids (*5*) and change in the kind of free fatty acids or in their concentration was observed.

The effect of heating fat at 100° C. in air was also studied. A wide-mouthed test tube, 5.5 cm. in diameter and 14.5 cm. deep, equipped with a standard taper 55/50 joint, was used as the heating chamber for 20 grams of fat. The use of a wide tube gave a high surface-to-volume ratio. Air at a flow rate of 2 liters per hour was first passed through a purification tower containing sodium carbonate admixed with silica gel, through a silica gel drying tower, and then across the surface of the heated fat and into a trap cooled in dry ice–isopropyl alcohol. No titratable acidity was found in the volatile fraction. The fat residues were analyzed for free fatty acids (Table I).

Determination of Carbonyl Compounds in Beef and Pork Fat. Samples of beef and pork fat were taken for "carbonyl" analyses from the same batches of fat used in the fatty acid studies and subjected to the same heat treatments. Volatile carbonyl compounds in the total condensate, from the samples heated under vacuum, were converted to their 2,4-dinitrophenylhydrazones (DNPH's) by the addition of 20 ml. of a 2,4-dinitrophenylhydrazine saturated solution of 2*N* hydrochloric acid. Steam-volatile carbonyl compounds present in the fat residues were steam distilled at a rate of 6 ml. per minute into an ice-cooled flask, containing 50 ml. of a similar mixture.

The heat treatment in air was like the procedure described for acids; the trapping solutions for the volatile carbonyl compounds were, however, different. Air, at a rate of 2 liters per hour, was first pulled through a purification trap containing 20 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid; secondly, through a silica gel drying tower; and then through the heat changer containing the fat. The final trapping solution was maintained at 0° to 4° C. and contained 20 ml. of a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid. The steam-volatile carbonyl compounds in the residue were converted to DNPH's by steam distilling into a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid. The paper chro-

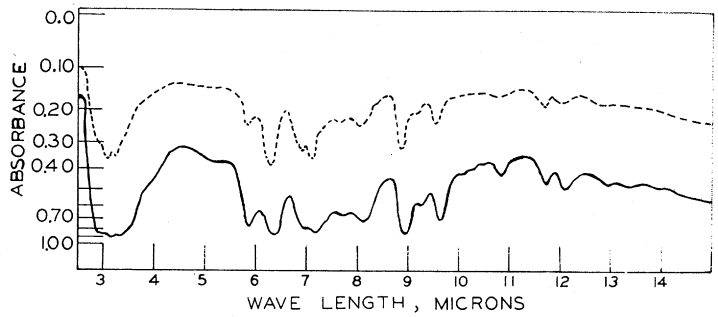

Figure 1. Infrared spectrum of fraction I obtained from lean beef

--- Beef
—— Pork

Table I. Free Fatty Acids Found in Beef and Pork Fat

(Before and after heating in air for 4 hours at 100° C.)

Acid	Beef, Mg./Gram		Pork, Mg./Gram	
	Before	After	Before	After
Lauric	0.04	0.16	0.08	0.56
Myristic	0.49	2.04	0.54	1.39
Tetradecenoic[a]	0.36	2.24	..	..
Pentadecanoic[a]	0.06	0.15	..	..
Palmitic	2.24	4.91	2.89	3.62
Hexadecenoic[a]	1.31	4.98	1.64	3.45
Heptadecenoic[a]	0.19	0.44	..	..
Stearic	0.96	1.37	0.77	3.21
Oleic	9.24	19.74	17.01	28.52
Linoleic	0.58	1.34	5.45	13.27
Linolenic	..	..	1.04	1.45
Total	15.47	37.37	29.42	55.47

[a] Structure assigned on basis of retention volumes; factors for converting areas to concentrations obtained by interpolation from known factors for neighboring acids.

matographic methods used in separating, and quantitatively estimating the monocarbonyl DNPH's were described by Gaddis and Ellis (*3, 4*).

Flavor Precursors. Possible Precursor Systems. The technique developed for vacuum pyrolysis of the lyophilized powder containing lean beef flavor precursors was applied to the following systems: gelatin; gelatin plus glucose; egg albumin; egg albumin plus glucose; and egg albumin, glucose, and lactic acid.

The odors of the total condensate were noted for each system. No analytical data were obtained.

Dialysis Experiments. One hundred grams of lean ground beef were blended with 350 ml. of water. The resulting slurry was centrifuged and the supernatant liquid decanted through Whatman No. 1 paper to remove fat particles. The filtrate was concentrated by freeze-drying to approximately 75 ml. The liquid was then dialyzed against an equal volume of water through a seamless cellulose membrane with gentle shaking, overnight, in a cold room. This dialyzate was lyophilized to yield a white, fluffy powder. The contents of the dialysis tube were further dialyzed, overnight, against cold, running water, and the solution remaining in the tube was examined separately.

In another experiment, 75 ml. of concentrated beef extract were placed in a 100-ml. glass-stoppered graduate and dialyzed against 40 grams of Dowex 50, prepared by the method of Hunter, Houston, and Kester (*9*), and placed in the dialysis tube. The amino acids that dialyzed into the tube were absorbed on the ion exchange resin over a period of 60 hours at 4° C. The resin was then washed with distilled water and poured into a "chromatographic" column, and the absorbed amino acids were eluted with 350 ml. of 5*N* ammonium hydroxide. The first 75 ml. of eluate were discarded and the remainder was concentrated under vacuum to about 100 ml.

Table II. Carbonyl Compounds Isolated from Beef and Pork Fat Heated in Air at 100° C.

Fraction	Class	Concn. of Classes, μmoles Carbonyl/10 Grams Fat	Compounds Isolated from Each Class
		Beef Fat	
Volatile	Alkanal	0.039	Acetone[a,b] Propanal
	2-Enal	0.072	Acetaldehyde[c]
Steam-distilled fraction	Alkanal	0.070	Hexanal[b] Nonanal
	2-Enals	0.028	Hepta-2-en-1-al[d] Octa-2-en-1-al[d] Nona-2-en-1-al Deca-2-en-1-al[b] Undeca-2-en-1-al
	2,4-Dienals	0.021	Deca-2,4-dienal
		Pork Fat	
Volatiles	Alkanals	0.27	Acetone[a] Propanal Hexanal[b] Octanal Nonanal
	2-Enals	0.06	Acetaldehyde[c] Hepta-2-en-1-al[d] Octa-2-en-1-al[d] Nona-2-en-1-al[d] Deca-2-en-1-al[d] Undeca-2-en-1-al[d]
	2,4-Dienals	..	Deca-2,4-dienal[d]
Steam-distilled fractions	Alkanals	0.24	Hexanal[b] Octanal Nonanal Undecanal
	2-Enals	0.12	Hepta-2-en-1-al Octa-2-en-1-al Nona-2-en-1-al Deca-2-en-1-al Undeca-2-en-1-al
	2,4-Dienals	0.083	Hepta-2,4-dienal Nona-2,4-dienal Deca-2,4-dienal[b]

[a] Acetone isolated with alkanals.
[b] Major component in class.
[c] Acetaldehyde isolated with 2-enals.
[d] Trace amounts (estimated at less than 0.005 μmole).

Results and Discussion

The desirable aroma of fraction I obtained from beef disappeared when efforts at fractionation (regardless of technique) were made. Paper chromatography was selected as the least destructive separation procedure and was used to study I.

The major differences in the infrared spectra of the fractions separated from I by paper chromatography were observed at 6, 7, and 12 microns. An examination of the spectra-structure correlation in the infrared region (*1*) strongly suggested that I was, to a large extent, a mixture of an organic acid and its ammonium salt. This conclusion was further confirmed when titration curves for I exhibited buffering action in the pH regions 3.0 to 5.0 and 8.0 to 10.1, and analyses showed that all the nitrogen was labile. Kjeldahl nitrogen values were 6.06%. Lactic acid was a likely possibility for the unknown acid, since it is the end product of glycolysis. Qualitative tests (*2*) confirmed the presence of lactic acid, and the quantitative results indicated that approximately 87% of I was lactic acid. If one assumes that the labile nitrogen is present as ammonium lactate, then over 93% of I is accounted for. As I was found to be optically active, it was concluded that optically active lactic acid, presumably an end product of glycolysis, accounted for approximately 90% of fraction I and that approximately 5% of ammonia, formed during the pyrolysis of beef powder, was fortuitously trapped in the acid. In addition, at least one unknown material, with a strong ultraviolet absorbance at 290 to 295 mμ and possibly responsible for the "meaty" aroma, was also present in fraction I.

The odor characteristics of the corresponding fraction I from lean pork were identical to that from beef I. The infrared spectrum of fraction I, obtained from lean pork, was qualitatively identical to that of fraction I from lean beef. The ultraviolet spectrum for both beef I and pork I exhibited an identical absorbance peak at 290 to 295 mμ. The paper chromatographic separation of pork I yielded fractions identical in R_f values and of approximately the same concentration as the corresponding fractions from beef I. Nitrogen was again shown to be present in the form of labile ammonia, and approximately 85% of pork I was found to be lactic acid. The most volatile fraction obtained from lean pork was identical in odor to the corresponding fraction from lean beef. Carbonyl compounds found in this fraction were acetone, acetaldehyde, and formaldehyde. The only basic compound isolated was ammonia. Hydrogen sulfide and carbon dioxide were

odor detected. Reducing sugars, present in muscle, could react with protein and possibly produce the observed aromas. A mixture of gelatin plus glucose was vacuum-pyrolyzed; the results were negative. Gelatin is, however, low in sulfur-containing amino acids, and so the same experiments were repeated with soluble egg albumin powder and with egg albumin plus glucose. The results were again negative. Since lactic acid was isolated in appreciable quantity and pH could have a profound effect on the results of heat treatment (pH of an initial cold water extract on ground beef was approximately 5.5), a model system, consisting of 10 grams of egg albumin, 2 grams of glucose, and 100 mg. of lactic acid, was prepared and treated in the same fashion as the lyophilized lean meat extracts. Some odor was obtained, indicating the lactic acid may be important in developing flavor in cooked meat. However, this odor did not resemble the odor "profile" obtained from the meat powders. Relaxation of rigor with time results in the production of a more desirable flavor than that associated with fresh beef. It may be that as glycolysis continues the increase in lactic acid concentrations may result in this better flavor.

Because the protein systems heated were apparently not the flavor precursors sought, attention was directed to the low molecular weight fraction present in the water extract of lean meat. Dialysis experiments indicated that the material passing through the membrane contained some of the flavor precursors of lean beef. When the white, fluffy powder obtained from this dialyzate by freeze-drying was carried through the vacuum pyrolysis and fractionation procedure applied to the lyophilized water extracts of lean beef and lean pork, a fraction very similar to beef I and pork I was isolated. However, when the free amino acids were separated from a similar dialyzate and heated alone, at both pH 5.5 and pH 8.0, no recognizable meaty odors were obtained. The odors were, in fact, classified as unpleasant, indicating that the free amino acids as such were not flavor precursors.

Conclusions

The identity of the compounds isolated from lean beef and lean pork, as well as the marked resemblance in chromatographic and spectrophotometric behavior of fractions not completely characterized, leads to the conclusion that a similar basic meaty flavor is obtained on heating the lean of beef and pork.

The flavor differences that exist in pork and beef may have their origins in the fat portions of these meats. The fat may not only produce different flavor compounds in different ratios, but perhaps also act as a storage depot for lipide-soluble foreign compounds that, on heating, also contribute to flavor.

Examination of possible precursor systems for the origin of lean meat flavor indicated that the flavor precursors may well be the low molecular weight, water-soluble fractions of lean meats. These produce the characteristic flavor of lean meat, presumably by some kind of interaction between amino acids and the low molecular weight carbohydrates and polypeptides present, and no single compound or class of compounds is responsible for cooked "meat" flavor.

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